DISCUSSION

Results obtained in previous work (Levvy, 1946) suggested that some arsine entering the blood stream may escape reaction with the red cells long enough to be fixed by the body tissues. From the experiments described above, it appears that if it does reach these organs *in vivo*, arsine will have a toxic action on the liver and kidneys. Under the conditions of the experiments, arsine was as toxic as arsenite for kidney tissue, and considerably more so for liver. The action of unchanged arsine in solution in the blood plasma on the liver, and perhaps also other organs, may thus explain, in part at least, the high toxicity of this gas when it is rapidly inhaled. The change in the appearance of liver slices when treated with arsine suggests that a reaction with the gas analogous to the characteristic one which occurs with haemoglobin may take place in the cells of this organ. An examination of the effects of poisoning *in vivo* with arsine on certain enzyme systems, besides yielding valuable information regarding the mode of action, might provide further evidence for the view that it has a specific action on body cells. It is of interest to note that arsenite, which did not change the appearance of liver slices, had the same toxicity for this tissue as for kidney.

If one accepts the postulate that the toxicity of arsine for the whole animal is in part due to effects produced by the gas itself in the body tissues, the decrease in its toxicity with decreasing concentration inhaled (Levvy, 1946) becomes readily explicable. As the rate of inhalation falls off, more of the gas will be fixed by the erythrocytes before it can be transported round the body, and thus transformed to less toxic arsenic derivatives. The nature of the arsenic-containing products of the reaction in blood has been studied by Graham, Crawford & Marrian (1946).

The technique described above may find general application in experiments in which it is desired to study the effect of known concentrations in solution of substances which are normally gases on the metabolism of surviving tissues.

SUMMARY

1. A technique was devised for treating surviving liver and kidney slices in absence of any gas phase with arsine solutions of known concentration.

2. The effect of arsine in reducing the oxygen uptake of rat kidney was identical to that of arsenite.

3. With liver, a 50% inhibition in the oxygen uptake was obtained with a lower concentration of arsine than of arsenite.

4. The bearing of these findings on the toxicity of arsine for the whole animal is discussed.

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Histidine Deficiency in the Rat and its Effect on the Carnosine and Anserine Content of Muscle

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The original purpose of the work described in this paper was to establish whether histidine was essential to the maintenance of body weight in the adolescent and adult rat and to investigate the symptoms of such deficiency if it occurred. Since our work has been completed, two papers dealing with the same subject have appeared. Albanese & Frankston (1945) have shown that histidine is required in the diet both by young and adult rats, if body weight is to be maintained. Maun, Cahill & Davis (1946) confirming this finding have noted a slight reduction in haemoglobin and plasma protein

content, a definite atrophy of the thymus and delayed spermatogenesis in histidine-deficient animals. Our results confirm fully those obtained by the American workers as far as loss of weight, anaemia and hypoproteinaemia are concerned and the slight differences observed may be mainly due to the longer period of histidine privation employed in many of our experiments.

One aspect of our work, however, has not been touched upon by the American workers and will be described in some detail. A deficiency of an essential amino-acid in the diet will in the first place cause a disturbance in the synthesis of protein in the body and so far as the function of an amino-acid is confined to that of building-material for protein synthesis, a dietary deficiency will produce unspecific symptoms which can only vary quantitatively with different amino-acids. But if one amino-acid has a function not shared by others, as, for example, of being the precursor of some particular body-constituent, specific changes either in the composition of tissues and/or in bodily functions may be expected to occur if such an amino-acid is omitted from the diet. It was shown by Schoenheimer, Rittenberg & Keston (1939) that whilst the amino-N of histidine was exchanged readily in the body with dietary $N^{15}H_3$, the nitrogen of the iminazole nucleus was quite inert. From this observation and the results reported in another paper (Neuberger & Webster, 1946) it can be concluded that the rat cannot synthesize this heterocyclic nucleus at all and it can be assumed that all body constituents possessing an iminazole nucleus are derived from dietary histidine. There are at least four substances besides histidine itself occurring in mammalian tissues which have an iminazole or a modified iminazole structure: carnosine, anserine, histamine and ergothioneine. There is considerable doubt as to whether ergothioneine is a normal body constituent and it is most probably entirely of exogenous origin (Potter & Franke, 1934). Histamine is present in minute quantities only and its estimation by either chemical or biological methods appeared to be of questionable value in view of the rather unspecific nature of the methods available. We have therefore directed our attention to the determination of the two β-alanine peptides of histidine and methylhistidine respectively, which occur in all mammalian muscles in considerable quantities and represent the largest store of histidine derivatives in the body apart from the even greater fraction of the amino-acid which is bound in proteins.

Carnosine is usually estimated by the Pauly reaction and, though this reaction is not very specific, it has been shown by Hunter (1922) that about 95% of the colour given by muscle extracts containing large amounts of carnosine, is in fact due to this peptide. Rat muscles contain very little carnosine, but relatively large quantities of anserine which have been identified by isolation (Wolff & Wilson, 1932). Anserine and *l*-methylhistidine do not give the Pauly reaction and no method for the direct estimation for either of these substances is available. Zapp & Wilson (1938) assumed that the sum of the two peptides is equivalent to the increase of α-amino-nitrogen obtained on hydrolyzing the material precipitable by mercuric acetate at neutral reaction. Carnosine was estimated by the diazo reaction and anserine by difference. The authors themselves have drawn attention to the possibility that other peptides precipitable by mercury may be present in muscle extracts and may give rise to a considerable error. Bate Smith (1939) suggested that the residual buffering power of rigor muscle, i.e. the difference between total buffering power and that due to protein, phosphate, carnosine and lactic acid, could give a 'rough guide to the amount of anserine present in muscle'. Neither of these two methods appeared to be sufficiently specific for our purpose, and the possibility of using the concentration of β-alanine as a measure of the combined anserine and carnosine content was therefore investigated. Several microbiological methods for the estimation of β-alanine have been described; the method used in our work was based on that described by Schenck & du Vigneaud (1944). In order to assess the suitability of this method for our particular purpose, it was necessary to investigate the distribution of β-alanine in different tissues. Pantothenic acid which also contains β-alanine has a wide distribution in animal tissues, and this and the possible presence of other so far unknown compounds containing β-alanine could invalidate this method as a measure of carnosine and anserine content of the body. Another point which had to be investigated was the interference with the assay method used by protein degradation products present in the hydrolysate.

EXPERIMENTAL

Litters of black and white rats of the Institute strain with initial weights varying in two separate series from 180–200 and from 93–111 g. respectively were used. Weights were recorded every second day and food consumption was also measured.

Diet. The dietary protein was given in the form of amino-acids and the composition of the amino-acid mixture and of the diet as a whole was identical with that described in an earlier paper (Neuberger & Webster, 1946). Controls were given in addition 35 mg. *l*-histidine/day. The intake of amino-acids in both groups was kept constant as far as possible and varied between 1·3 and 1·4 g. The intake of the non-protein part of the diet varied with the appetite. Animals on a 'non-protein' diet were not given amino-acids, but otherwise the same diet as above.

In order to investigate the effect of other variations in the diet small groups of animals were given 'casein',

tryptophan-deficient and stock diets respectively. The casein diet contained 16% of casein and the tryptophan-deficient diet contained 16% of zein supplemented with 50 mg. of *l*(+)-lysine hydrochloride/day. In these experiments the protein intake varied with the food intake. The other components of the diet were given in the same proportions as in the earlier experiments.

Haemoglobin and protein content of plasma and liver. The methods were the same as those used in an investigation on lysine deficiency (Gillespie, Neuberger & Webster, 1945).

Estimation of β-alanine. β-Alanine estimations were carried out both on hydrolysates of whole animals or separate organs and on deproteinized extracts of muscles and other tissues. For the hydrolysis of whole rats the directions of Schenck & du Vigneaud (1944) were followed. Muscle extracts were prepared by extracting ground muscles three times with water at 70° for three periods of 20 min., the combined extracts were filtered through muslin, made up to a known volume after addition of one or two drops of 25% acetic acid and heated gently to boiling. The coagulated protein was filtered off. A sample of this solution was used for the estimation of carnosine. For the determination of β-alanine 2 vol. of the extract were mixed with 1 vol. of 37% HCl (w/w) and the solution was then heated for 2 hr. at 100°, taken to dryness *in vacuo*, the residue dissolved in water and made up to volume.

The β-alanine contents of other tissues were determined either on hydrolyzed whole organs or on deproteinized extracts. In the latter case whole organs were ground in the Potter-Elvehjem homogenizer, suspended in 20 vol. of water made just acid to methyl orange with acetic acid, and heated at 70° for 20 min. This extract was then filtered and hydrolyzed in the same manner as described for muscle. When the complete hydrolyzed tissue was desired, organs were cut up with scissors and boiled under reflux for 10 hr. after addition of 20 times their weight of 20% HCl (w/w). The further treatment followed exactly that described above for muscle.

Tissue extracts or hydrolysates were assayed for β-alanine by a yeast-growth method which was very similar to that used by Schenck & du Vigneaud (1944) using the Gebruder Mayer strain of *Saccharomyces cerevisiae* (National Type Collection No. 4614). This strain, however, cannot utilize carnosine instead of β-alanine, in contrast to the strain used by the American workers. It was also found that with the medium used by Schenck & du Vigneaud the slope of the growth curve was steeper with hydrolysates of rat tissues than with β-alanine solutions and this appeared to be due to the presence of growth factors other than β-alanine in the extracts. The addition of an acid hydrolysate of vitamin-free casein to a final concentration of 0·04% to the basal medium removed this discrepancy. Growth was estimated by measuring the turbidity of the yeast suspension with a Spekker absorptiometer with a neutral screen of 1·5 density. Growth was linearly proportional to the β-alanine concentration over a relatively narrow range and this part of the curve was fairly steep. Care was taken to dilute samples in such a way as to obtain readings well within the linear range of the standard curve.

Inhibition of yeast growth by other tissue components. In most of our experiments protein-free extracts of muscles were used; such extracts are rich in β-alanine and contain presumably only small amounts of other substances having either a stimulating or inhibitory effect on the growth of yeast. Recovery of added β-alanine with such extracts is quantitative within the limits of reproducibility of the method which is about 5%. When whole tissues, however, are hydrolyzed and particularly tissues containing only traces of β-alanine, considerable inhibition by products of hydrolysis occurs. This is shown by the fact that assay of whole hydrolyzed tissues such as lungs, kidney and blood indicated complete or almost complete absence of β-alanine, whilst protein-free extracts of these organs appeared to contain small, but measurable amounts of this amino-acid. This inhibition by tissue components has already been noted by Schenck & du Vigneaud (1944) in the case of liver and may be due to products of protein hydrolysis. Such an interpretation is supported by the work of Nielsen, Hartelius & Johansen (1944) who found that nearly all α-amino-acids counteract the stimulation of growth of *S. cerevisiae* produced by β-alanine. This inhibition becomes apparent when the ratio of α-amino-acid to β-alanine exceeds the value of 300. In our experiments the addition of a casein hydrolysate to both standard and test solutions makes allowance for this source of error and in protein-free extracts of muscle and liver, tissues which are rich in β-alanine, inhibition caused by α-amino-acids is not likely to be of importance. In hydrolysates of whole rats, the ratio of α-amino-acids to β-alanine is less favourable, but even in this case the amount of nitrogen added is smaller than that present in the basal medium and results can be considered fairly reliable, unless the β-alanine content of the hydrolysate is very low. Recovery of β-alanine added to hydrolysates of whole rats was quite satisfactory, but such a test is not very stringent. The addition of β-alanine to a hydrolysate increases the ratio of β-alanine to α-amino-acids and conditions are not strictly comparable to those obtaining in the hydrolysate itself. Evidence was in fact obtained indicating that the values of β-alanine found for whole rats may be too low by about 20%. Values obtained with hydrolysates of whole organs other than muscle and possibly liver are wholly unreliable.

There is of course the possibility, as in all microbiological assays applied to complex materials, that an inhibitory or stimulating effect on the growth of yeast may be exerted by other compounds present in the mixtures. We have no data which would definitely exclude such a possibility, though it appears unlikely in view of the good recovery of added β-alanine to these extracts. As will be shown later, the β-alanine values obtained for muscle are of the expected order if it is assumed that β-alanine is entirely derived from anserine and carnosine. Moreover, the β-alanine contents of whole rats agreed quite well with values calculated on the assumption that nearly the whole β-alanine of the body is contained in muscle. It is concluded therefore that the results obtained are reasonably reliable and are strictly comparable.

Estimation of carnosine. Carnosine was determined colorimetrically by the Pauly reaction with diazotized sulphanilic acid with carnosine nitrate as a standard. All the various published modifications of this method have been tried, but with rat muscles which contain only small amounts of carnosine a yellow colour was always obtained which must be due to a substance other than carnosine; this was particularly noticeable in extracts of very low carnosine content. In an attempt to overcome this difficulty which has been experienced by other workers (Eggleton & Eggleton, 1933) the method of Edlbacher, Baur, Staehelin & Zeller (1941) for the estimation of histidine was adapted to that of carnosine. In this method diazotized *p*-chloro-aniline

is used and the resulting pigment being soluble in butanol can be readily extracted from aqueous solution. It was found, however, that with rat muscle extracts the interfering pigment was also extracted into the organic solvent.

The procedure finally adopted was as follows. The cooled diazotized sulphanilic acid was added to the solution being tested and to the carnosine standard, the exact quantities and concentrations of reagents being similar to those used by Meschkowa (1936). After 10–15 min., when the colour had reached its maximum, the two solutions were compared in a colorimeter with a Wratten no. 75 filter. This, as pointed out by Eggleton & Eggleton (1933) makes matching easier, but does not necessarily avoid the error due to the substance giving the yellow colour. If about 5% of colour given by muscle extracts of horse or frog which are rich in carnosine is due to other substances (Hunter, 1922), this error must be considerably larger in rat muscles; it is believed therefore that the carnosine figures given in this paper are probably too high, but are nevertheless comparable.

RESULTS

Changes of body weight. Rats on a histidine-deficient diet cannot maintain their body weight (Table 1). The rate of loss is very marked in the first and second weeks of the deficiency, but becomes less pronounced in the later stages. The actual weight losses are smaller than those observed in deficiencies of some other essential amino-acids investigated by us such as methionine.

Haemoglobin content. Histidine deficiency leads to a mild anaemia as shown in Table 1 which indicates that the haemoglobin figures obtained for deficient animals are about 20–25% below those considered normal for rats of similar age. The anaemia found in histidine deficiency is similar to or slightly less severe than that observed in lysine deficiency (Gillespie *et al.* 1945).

Plasma protein content. Lack of histidine in the diet produces a marked reduction of plasma proteins, the average content after varying periods of deficiency being about 5·3%. The corresponding figure for lysine deficiency was found to be 5·2%, whilst in methionine deficiency in younger animals an average figure of 3·6% was obtained (Glynn, Himsworth & Neuberger, 1945). On a protein-free diet, values of 3·0–3·2% were obtained (Table 1).

Changes in the composition of the liver. Table 2 records the crude composition of the liver of animals which had been on a diet deficient in histidine for varying periods; corresponding figures are also given for animals on non-protein lysine-deficient diets, and

Table 1. *Changes of body weight, haemoglobin and plasma protein contents of rats receiving amino-acid diets with and without histidine and of rats on a non-protein diet*

(The letters A, B, etc. refer to litters; litter-mates were, as far as possible, evenly distributed over the different dietary groups. H indicates diet with histidine, HD, diet without histidine, and NP, non-protein diet.)

Rat no.	Body wt. Initial (g.)	Body wt. Final (g.)	Days on diet	Diet	Blood haemoglobin (g./100 ml.)	Plasma protein (%)	Average daily food consumption (g.)
B 1	157	113	54	HD	13·8	5·7	7·5
B 6	134	101	41	HD	12·4	—	6·4
C 1	207	156	64	HD	12·3	5·4	8·6
C 3	145	98	62	HD	—	—	6·5
C 4	140	92	63	HD	11·8	5·3	6·8
X 1	111	81	33	HD	13·0	5·0	5·2
Z 4	108	73	33	HD	12·4	5·0	5·5
Y 3	104	76	33	HD	13·0	5·0	5·2
Z 6	110	76	33	HD	13·3	4·8	5·4
B 2	150	233	54	H	16·5	7·0	14·0
A 1	93	55	42	NP	9·8	3·0	8·2
A 3	105	58	42	NP	11·5	3·2	8·4
Z 5	125	75	42	NP	10·2	3·2	5·2

Table 2. *Nitrogen, fat and water contents of livers of rats on different diets*

(All the figures, with the exception of those for liver water which are expressed in terms of mg./100 g. of body weight, refer to average values; the figures in brackets give the standard error of the mean.)

No. of animals used	Diet	Days on diet	Liver-N	Liver fat	Liver water
4	Histidine-deficient	33	114 (±5)	60 (± 7)	73·4 (±0·1)
5	Histidine-deficient	41–63	90 (±3)	56 (± 8)	73·6 (±0·2)
5	Non-protein	42	80 (±7)	197 (±25)	76·2 (±0·2)
5	Restricted stock diet	45	98 (±2)	65 (±15)	73·5 (±0·3)
7	Lysine-deficient	48	85 (±2)	160 (±35)	73·9 (±0·3)

of rats whose caloric intake was restricted in such a way as to keep their body weight about constant. The corrected water content, i.e. g. of water/100 g. fat-free liver was definitely elevated in the animals which had subsisted on a protein-free diet, was possibly slightly increased in lysine deficiency, but was definitely normal in histidine-deficient animals. The fat content of the livers was normal or even below normal in all the groups investigated. It is clear that diets deficient in the three essential amino-acids lysine, histidine or methionine (Himsworth & Glynn, 1945) do not produce fatty livers, if sufficient choline is given.

If a histidine-deficient diet is given for 33 days the total liver-N is only slightly reduced, i.e. on the average by about 10% (Table 2). Prolonged deficiency appears to lead to a much more severe reduction. The slight reduction in the first five weeks may be entirely due to the reduced caloric intake of the diet (Harrison & Long, 1945; Kosterlitz & Campbell, 1945).

The β-alanine content of tissues other than muscle. Table 3 shows the concentrations of β-alanine in several organs as found by ourselves in hydrolyzed

Table 3. *The β-alanine contents of different organs of the rat*

(In these experiments the organs of three rats of a total wt. of 374 g. were pooled and treated as described in the text.)

Organ	Amount of tissue taken (g.)	β-Alanine found (mg.)	β-Alanine in tissue (mg./100 g.)	Pantothenic acid* in tissue (mg./100 g.)
Blood	9·2	0·017	0·19	—
Liver	19·1	1·43	7·5	7·3 –15·0
Kidney	3·4	0·034	1·0	3·2 – 3·4
Testis	6·2	0·028	0·45	—
Lung	3·0	0·010	0·35	0·85– 0·95
Heart	1·4	0·014	1·0	3·0 – 4·0
Gastro-intestinal tract	21·0	0·128	0·61	—
Brain	4·3	0·018	0·43	1·2
Combined muscles of hind-leg	25·0	15·25	61·0	0·45– 0·55
Combined muscles of shoulder	13·4	8·58	64·0	

* Figures given by Mitchell & Isbell (1942).

protein-free extracts and also the amounts of pantothenic acid as observed by Mitchell & Isbell (1942). With the exception of muscle there is a close correlation between the two sets of values and it is highly probable that most of the β-alanine present in the body apart from that found in muscular tissue forms part of the pantothenic acid molecule. The total amount of β-alanine present in tissues other than muscle is comparatively small (Table 3); it can be estimated that at least 95% of the total β-alanine of the rat is present in muscle. Such an estimate is also borne out by a comparison of the β-alanine contents of isolated muscles and that of whole rats respectively.

Table 4. *Carnosine and β-alanine contents of muscle extracts of rats on different diets*

Rat no.	Body wt. (g.)	Diet	Muscle content (mg./100 g.) of β-Alanine	Muscle content (mg./100 g.) of Carnosine
A 5	191	Stock diet	71	98
B 3	203	Stock diet	63	106
C 5	161	Stock diet	82	76
C 8	155	Stock diet	62	74
A 4	206	Casein	86	109
B 4	198	Casein	76	77
C 6	272	Casein	111	116
Z 1	180	Casein	70	69
B 2	233	Complete amino-acid	104	103
A 5	123	Non-protein	97	75
A 1	55	Non-protein	85	70
Z 5	75	Non-protein	105	95
B 7	132	Tryptophan-deficient	80	56
C 7	105	Tryptophan-deficient	85	61
Z 1	106	Restricted stock diet	80	77
Z 2	95	Restricted stock diet	91	84
B 1	113	Histidine-deficient	62	26
B 6	101	Histidine-deficient	65	29
C 1	125	Histidine-deficient	59	28
C 3	120	Histidine-deficient	63	31
X 1	81	Histidine-deficient	57	35
Z 4	73	Histidine-deficient	68	34

Table 4 shows the values for the mixed muscles of the fore- and hind-legs of rats on normal diets which varied between 55 and 115 mg. of β-alanine/100 g. of muscle with an average value of about 86 mg. If it is assumed that 95% of all the β-alanine is contained in muscle and that the muscles of the leg are representative of muscular tissue generally, then the whole β-alanine content of the body can be calculated, if the proportion of muscle relative to body weight is known. Estimates for this ratio made by different authors vary between 30 and 40%, and we may use for our calculation a value of 35%. The expected β-alanine content of the whole body on this basis is 28 mg./100 g. of body weight. The average value

Table 5. *β-Alanine contents of whole rats on different diets*

Rat no.	Wt.	Diet	Amount of β-alanine in rat (mg./100 g.)
D 1	110	Stock diet	19
E 3	200	Stock diet	31
D 3	55	Stock diet	24
D 4	98	Stock diet	23
B 1	113	Histidine-deficient	23
C 4	98	Histidine-deficient	30
Y 3	76	Histidine-deficient	20
Z 6	76	Histidine-deficient	20

found by Schenck & du Vigneaud (1944) and also by ourselves is about 23 mg.; the agreement is as good as can be expected in view of the many uncertainties involved.

The β-alanine content of muscle extracts. The amount of β-alanine in muscle extracts after mild hydrolysis is used in the present work as a measure of the concentration of the two peptides, carnosine and anserine. The assumption implied is that the amount of free β-alanine and the concentration of other compounds liberating β-alanine is negligible compared with that of carnosine and anserine. The validity of this assumption could not be tested directly, since no other reliable method for the estimation of β-alanine or for that of methylhistidine is at present available. But the following facts appear to justify this assumption. The amount of pantothenic acid in muscle is extremely small (Mitchell & Isbell, 1942) and only traces of free β-alanine could be found in fresh muscle extracts. No compound containing β-alanine other than carnosine, anserine and pantothenic acid has as yet been obtained from biological sources. Moreover, a comparison between the values of carnosine and anserine obtained by the assay of β-alanine and those of other workers who used different methods suggests that the method we have been using certainly does not over-estimate the concentration of these peptides. If the results of Table 4 are recalculated in terms of anserine the average amount in 100 g. of muscle is found to be about 230 mg. whilst the highest and lowest values are 310 and 140 mg. respectively. Zapp & Wilson (1933) found, with a rather unspecific method, a value of 360 mg./100 g. for the combined anserine and carnosine content of rat muscle, whilst Bate Smith (1939) calculated from the buffering curves of muscle extracts anserine contents varying between 160 and 310 mg./100 g. in good agreement with our figures.

β-Alanine and carnosine contents of muscles of rats and the effect of different diets. Table 4 shows that the β-alanine contents of muscles of rats supplied with a satisfactory diet (casein- and stock-diet groups) varied between rather wide limits. This agrees with the findings of other workers who studied the concentrations of carnosine in the muscles of species with low anserine and high carnosine contents (Hunter, 1924; Mitsuda, 1923; Eggleton & Eggleton, 1933). These authors found a wide variation in the figures obtained for the carnosine content of different muscles from the same animals and also for similarly placed muscles taken from different individuals of the same species. Removal of protein or of an essential amino-acid, like tryptophan, from the diet does not significantly affect the values, nor has restriction of caloric intake leading to loss of weight any apparent effect. The values for β-alanine found for muscles of rats which had been on a histidine-deficient diet for varying periods are near the lower limit of the normal range. It is doubtful, however, whether this slight apparent reduction is statistically significant in view of the wide variation in normal animals and the small number of data at our disposal. It can be concluded that at least in older animals prolonged dietary deficiency either does not lead to a change of the β-alanine and therefore of the combined carnosine and anserine content of muscle at all or leads only to a slight reduction which on the average does not exceed 25 %. The carnosine values appear to be definitely reduced in the histidine-deficient animals, but the quantitative significance of this finding is somewhat uncertain in view of the unspecific character of the method and the fact that carnosine has not to our knowledge been actually isolated from rat muscles.

It was found, however, that if animals are put on a histidine-deficient diet soon after weaning the β-alanine content decreases considerably to values of below 20 mg./100 g. and the substance(s) giving rise to the colour reaction with the diazo reagent disappear(s) almost completely from muscle extracts. It is doubtful if this finding can be attributed to histidine deficiency, since the weight of the animals is reduced to about 30 g. and rats of such low body weight usually have low β-alanine and carnosine values.

The effect of different diets on the β-alanine contents of whole rats. The data presented in Table 5 support the conclusions drawn from the β-alanine values of muscle extracts discussed above. The few normal values are within the range of the figures given by Schenck & du Vigneaud (1944) for a larger series of animals of similar weight. Deficiency of histidine in the diet has no significant effect on the β-alanine content of the whole body.

DISCUSSION

The essential character of histidine as a dietary constituent. The results reported in this and in an earlier paper (Neuberger & Webster, 1946), together with the observations of Albanese & Frankston (1945) and those of Maun *et al.* (1946), show clearly that neither the young nor adult rat can maintain its body weight if histidine is not present in the diet. Though loss of weight as such does not necessarily indicate loss of protein, a decrease amounting to a third or a fourth of the initial body weight, as was found in our experiments, must involve loss of body substance other than fat. It can be concluded that lack of histidine in the diet leads to loss of body-nitrogen and histidine must be classified as an amino-acid which is essential to the rat both for the purpose of growth and maintenance. The loss of weight is only partly due to the decreased food intake, since animals receiving a diet of a caloric value identical with that consumed by histidine-deficient animals

maintained their weight fairly well (Maun *et al.*, 1946). However, Burroughs, Burroughs & Mitchell (1940) found in experiments lasting 6–9 days that rats could maintain nitrogen equilibrium on diets not containing histidine, although some of their animals lost weight. A possible explanation for this discrepancy may be found in experiments on another species.

Dogs supplied by vein with all essential amino-acids remain in positive nitrogen balance (Elman, Davey & Loo, 1941). If histidine is omitted from the infusion fluid, the balance still remains positive for several days; after that the excretion of nitrogen in the urine rises and a marked negative nitrogen balance is observed. Similar results were obtained by Madden, Anderson, Donovan & Whipple (1945) on dogs whose plasma proteins had been depleted by frequent bleedings, the red cells being returned to the system. It was found that the ten essential amino-acids can cause abundant plasma protein production and maintain a positive nitrogen balance in these dogs. If histidine was omitted, there was at first no apparent change, but after a week the nitrogen balance became negative. What is the reason for this delay in the rise of nitrogen excretion? One possibility is, that the body can draw for a time on amino-acid reserves other than protein, to supply endogenous losses. Though the amounts of free amino-acids in plasma and tissues are low, they may be sufficient to replace, at least for a short time, an amino-acid which is irreversibly degraded at a slow rate only. Or it may be suggested as an alternative explanation that the body can still produce proteins for a time by internal rearrangement, if an amino-acid which cannot be synthesized, is omitted from the diet. Histidine for example is present in fairly large quantities in some proteins like haemoglobin, histones and insulin, whilst its concentration is smaller in most other proteins. It would be possible for the body therefore to break down histidine-rich proteins and to utilize the free amino-acid for the production of protein of low histidine content. In this way, or simply by differences in the rate of formation of proteins with different histidine content, a positive nitrogen balance could be maintained for a few days; only later, when the equilibrium between the different proteins becomes too greatly disturbed, will the body be unable to utilize other amino-acids and urinary excretion of nitrogen will increase. Whatever the explanation of this phenomenon may be, it is clear that the apparently contradictory results of Burroughs *et al.* (1940) on the one hand, and the findings of other American workers and those reported here on the other, are very similar to the observations made on histidine-deficient dogs.

In man, positive nitrogen balance can be maintained for weeks, though histidine is not present in the diet (Rose, Haines, Johnson & Warner, 1944). It follows from what has been said already that such a result does not necessarily indicate that man can maintain weight or nitrogen balance indefinitely on a histidine-free diet.

General symptoms of histidine deficiency. The loss of weight, anaemia, hypoproteinaemia and relative decrease of liver protein reported in this paper and the atrophy of the thymus, vascularization and epithelial metaplasia of the cornea found by Maun *et al.* (1946) in histidine-deficient rats present a picture which is very similar to that found in most deficiencies of amino-acids so far investigated in detail. The lack of an essential amino-acid in the diet always reduces food consumption, and loss of appetite with a resulting reduction of caloric intake is part of the effect of the dietary deficiency. The American workers have, however, shown that most of these changes are found either not at all or only to a lesser degree in rats receiving an equi-caloric diet and they must therefore be ascribed not to general malnutrition but to an inhibition of protein synthesis. The anaemia found was rather mild and this is particularly remarkable in view of the high concentration of histidine in haemoglobin. The histidine-deficient animals had a healthy appearance and, altogether, pathological changes were less marked than those found in deficiencies of other essential amino-acids. This could be explained by a limited synthesis of histidine by the rat. Such an explanation is unlikely for reasons discussed above. It appears more probable that these differences in the severity of symptoms are due to a quantitative variation in the demand for the different amino-acids, which in turn depends on the rate of irreversible degradation, and which may vary within wide limits for different compounds.

The carnosine and anserine contents of muscles of histidine-deficient rats. The function of these two peptides which are so universally distributed in muscles is still obscure although it has been suggested that they may be required for the regulation of pH (Bate Smith, 1938). It had been hoped to reduce the concentration of these substances by producing a dietary deficiency of their presumed precursor, histidine, and to relate any functional changes in the muscle to the decreased concentrations of the β-alanine peptides. It has not been possible to achieve this purpose. Even prolonged histidine deficiency leading to a loss of a considerable proportion of the protein of the body did not materially alter the concentration of β-alanine peptides either in the whole body or in particular muscles. It is likely that the formation of carnosine and anserine competes with other reactions requiring histidine, such as the formation of protein or histamine, and the net result of a dietary deficiency of histidine is a general and fairly uniform reduction in all these synthetic

processes leading to a loss of body substance as a whole without altering the relative composition of the tissues.

Another possibility is that both the degradation and formation of these two peptides are very slow and that changes of their relative concentrations in the muscles of the rat will only be noticeable after periods of dietary deficiency even longer than were used in the present work. No information is available as to the speed of synthesis or hydrolysis of the peptide linkage of anserine and carnosine *in vivo*, except for the results of Schenck, Simmonds, Cohn, Stevens & du Vigneaud (1943). These authors indicate that methylation at least is a relatively slow process. They fed methionine containing deuterium in the methyl group, and found anserine which was isolated from the muscles to have only a low deuterium content.

In this connexion it must be pointed out that our failure to detect any marked changes in the concentration of anserine in the muscles of the rat with various dietary deficiencies is in striking contrast to the findings of Hunter (1925), who investigated the factors affecting the concentration of carnosine in the muscles of the cat. This author observed that the carnosine content of striated muscles can be lowered by starvation and then raised again by a meat diet. It is possible that the level of carnosine in muscle is much more suceptible to dietary changes and this is important in the cat, where the concentration of carnosine, at least in some muscles is normally quite high. In the rat anserine which may be metabolically more inert and not be greatly affected by dietary restrictions, predominates and the β-alanine content may therefore be almost normal, although the carnosine is reduced. Such an explanation which assumes that the methylation or rather demethylation of the iminazole-N is the slowest step in the metabolism of anserine is supported by the fact that the carnosine figures found for histidine-deficient rats were significantly lower than those obtained on rats reared on normal diets.

SUMMARY

1. It has been shown that rats require the presence of histidine in the diet for the maintenance of body weight. The histidine requirements of other species have also been discussed, and it is suggested that the organism may be able to maintain a positive nitrogen balance for a limited period, although an amino-acid which cannot be synthesized by the body is absent from the diet.

2. The general effects of this dietary deficiency were found to consist of anaemia, hypoproteinaemia and loss of weight. The mildness of the anaemia is stressed, and it is suggested that the general result of a histidine deficiency is inhibition of protein synthesis similar in character but milder in degree than that found in most other deficiencies of essential amino-acids. It is concluded that the irreversible degradation of histidine proceeds at a slow rate.

3. The microbiological estimation of β-alanine has been used to estimate the combined carnosine and anserine contents of muscle extracts and of whole rats. It has been shown that at least 95 % of the total β-alanine of the rat is present in protein-free extracts of muscles. Inhibition of the assay method by α-amino-acids has been considered and been found to affect the results under certain conditions.

4. Values for the carnosine and anserine contents of rat muscles were obtained which were similar, but on the whole lower than the figures found with other methods. Histidine deficiency was found to reduce the carnosine content quite appreciably, whilst the anserine values were either not affected at all, or only slightly. Possible explanations for these findings are discussed.

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Metabolic Processes in the Gastric Mucosa

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The metabolic properties of the digestive tract have received attention from time to time, notably those of certain parts of the intestines (Dickens & Weil-Malherbe, 1941); but there is little recent work on the respiration and glycolysis of the gastric tissues apart from Rosenthal & Lasnitzki's (1928) measurements on the mucous membrane. Extensive studies on the localization of peptic and hydrolytic enzymes were carried out by Linderstrøm-Lang and his associates (relevant papers quoted by Rask-Nielsen, 1944); recently the presence of carbonic anhydrase was demonstrated in the gastric mucous membrane by Davenport (1939) and the enzyme was extracted from this material and purified by Keilin & Mann (1940).

In the present study, rather than to pursue the investigation of enzymes concerned with digestive processes in the stomach, an attempt was made to gather information about the respiration and glycolysis of the mucosa and the other gastric components so as to establish the metabolic requirements of the healthy normal mucosa and to gain insight into conditions which may lead to pathological changes such as erosions and ulcerations.

Certain aspects of the metabolism of the gastric mucosa were examined in 1944 in the course of research concerned with the mechanism of systemic poisoning with mustard gas (Lutwak-Mann, 1946); these studies were undertaken on behalf of the Ministry of Supply and the results were summarized by Dixon & Needham (1946) but full details will be published later. A part of the present work was communicated to the Biochemical Society (Lutwak-Mann & Barrett, 1946).

EXPERIMENTAL

Material. Rats were chosen as experimental animals. The anatomy of the rat stomach, an account of which may be found in a paper by Berg (1942), differs somewhat from the human, but it is probable that findings based on the behaviour of the rat stomach will have general application.

Usually animals weighing 100–150 g., but also younger and older rats, were examined. The rats received a mixed stock diet except in some experiments on special diets mentioned later. They were killed by decapitation, bled, and the whole stomach removed and placed in saline. The methods of preparing the tissue for an experiment varied and as the results depend to some extent upon the technique employed, the details are given below.

Methods. Anaerobic acid production and O_2-consumption were measured in Barcroft manometers with side bulbs and gas outlets. Ringer-phosphate (Krebs, 1933) was used for aerobic experiments; phosphate was replaced by bicarbonate for the assay of acid under anaerobic conditions in an atmosphere of 95% N_2+5% CO_2. Unless otherwise stated, the volume of the fluid in the manometer cups was 2 ml. and the dry weight of the tissue between 8 and 10 mg. The concentration of glucose or other metabolites added to saline was 0·2%. Volatile substances, e.g. alcohols, were placed in equal amounts in both sides of the manometric vessels.

Lactic acid was determined by the method of Friedemann, Cotonio & Shaffer (1927), phosphate by the method of Fiske & Subbarow (1925), N was determined by means of the Kjeldahl modification of Parnas & Wagner (1921), the content of adenosinetriphosphate (ATP) by the method of Parnas & Lutwak-Mann (1935), polypeptide-N following the procedure of Burstein (1937), the content of glutathione and ascorbic acid according to Hopkins & Morgan (1936).

RESULTS

Anaerobic glycolysis

Gastric mucosa detached from the rest of the stomach wall

As the gastric mucous membrane does not lend itself to the application of the ordinary slice technique for manometric experiments, the following procedure was employed in the earlier stages of this work.

The stomach was opened along the greater curvature and very thoroughly rinsed in several changes of saline. It was